

<110> RIEPING, Mechthild THIERBACH, Georg

<120> Process for the fermentative preparation of L-amino acids using strains of the Enterobacteriaceae family.

<130> 218162US0X

<140> 10/076,416

<141> 2002-02-19

<150> DE 10112107.5

<151> 2001-03-14

<150> US 09/987541

<151> 2001-11-15

ŊΊ

<150> US 60/283612

<151> 2000-04-16

<150> US 60/248210

<151> 2000-11-15

<160> 12

<170> PatentIn version 3.1

	<210)>	1														
	<211	_>	1719														
	<212	2>	DNA														
	<213	3>	Esche	erich	nia d	coli											
	<220)>															
	<221	.>	CDS														
	<222	?>	(1).	. (17	16)												
	<223	3>															
	<400		1														
	_		caa Gln	_	Val	_	_			Ála				_	Ser	_	48
	1				5					10					15		
			aaa Lys	Arg					Thr					Asn			96
				20					25					30			
_			agt Ser										Met				144
,	1		35					40					45				
		Glu	gaa Glu				Phe					Glu					192
		50					55					60					
	Gly		ctg Leu			Cys					Gly					His	240
	65					70					75					80	
			aac Asn		Leu					Arg					Val		288
					85					90					95		
			gcc Ala	Āla					Ser	_			-	бĺу			336
				100					105	•				110			
	cag	gaa	acc	cac	cca	caa	gag	cta	ttc	cgc	gaa	tgt	agt	cac	tat	tgc	384



	Gl	n Gl	u Th 11	r Hi 5	s Pro	o Glr	n Glu	1 Let 120	ı Ph∈	e Ar	g Gl	u Cy	s Se:		s Ty	r Cys	
	ga Gl	g ct u Le 13	u va	t tc	c ago r Sei	c cco	g gag Glu 135	ı G1r	g ato n Ile	c cca Pro	a caa o Gli	a gt. n Va 14	l Leı	g gco ı Ala	g at	t gcc e Ala	432
	ato Met 145	- TT	c aaa g Lys	a gco	g gto a Val	g ctt Leu 150	l Asn	cgt Arg	ggc Gly	gtt Val	tco Ser 15	r Val	t gto l Val	c gto L Val	g tta Lei	a cca ı Pro 160	480
	ggo Gly	c gad / Asp	c gto Val	g gco L Ala	g tta Leu 165	ıга	cct Pro	gcg Ala	cca Pro	gaa Glu 170	ı Glj	g gca 7 Ala	a acc	ato Met	g cad His	c tgg s Trp	528
	tat Tyr	cat His	gcg Ala	g cca Pro 180) GII	cca Pro	gtc Val	gtg Val	acg Thr 185	Pro	gaa Glu	ı gaa ı Glı	a gaa 1 Glu	gag Glu 190	ı Leı	a cgc ı Arg	576
	aaa Lys	cto Leu	gcg Ala 195	GII	ctg Leu	ctg Leu	cgt Arg	tat Tyr 200	tcc Ser	agc Ser	aat Asn	ato Ile	gcc Ala 205	Leu	atç Met	tgt Cys	624
	ggc Gly	ago Ser 210	СТУ	tgc Cys	gcg Ala	Gly	gcg Ala 215	cat His	aaa Lys	gag Glu	tta Leu	gtt Val 220	Glu	ttt Phe	gcc Ala	ggg	672
	aaa Lys 225	att Ile	aaa Lys	gcg Ala	cct Pro	att Ile 230	gtt Val	cat His	gcc Ala	ctg Leu	cgc Arg 235	ggt Gly	aaa Lys	gaa Glu	cat His	gtc Val 240	720
a	1914	tac Tyr	gat Asp	aat Asn	ccg Pro 245	tat Tyr	gat Asp	gtt Val	gga Gly	atg Met 250	acc Thr	Gly	tta Leu	atc Ile	ggc Gly 255	ttc Phe	768
	tcg Ser	tca Ser	ggt Gly	ttc Phe 260	cat His	acc Thr	atg Met	atg Met	aac Asn 265	gcc Ala	gac Asp	acg Thr	tta Leu	gtg Val 270	cta Leu	ctc Leu	816
	ggc Gly	acg Thr	caa Gln 275	ttt Phe	ccc Pro	tac Tyr	Arg	gcc Ala 280	ttc Phe	tac Tyr	ccg Pro	acc Thr	gat Asp 285	gcc Ala	aaa Lys	atc Ile	864
	att Ile	cag Gln 290	att Ile	gat Asp	atc Ile	Asn	cca Pro 295	gcc Ala	agc Ser	atc Ile	ggc Gly	gct Ala 300	cac His	agc Ser	aag Lys	gtg Val	912
	gat Asp 305	atg Met	gca Ala	ctg Leu	gtc Val	ggc Gly 310	gat Asp	atc Ile	aag Lys	Ser	act Thr 315	ctg Leu	cgt Arg	gca Ala	ttg Leu	ctt Leu 320	960
	cca	ttg	gtg	gaa	gaa	aaa	gcc (gat	cgc .	aag	ttt	ctg	gat	aaa	gcg	ctg	1008

• ,	•	

	Pro	Leu	Val	Glu	Glu 325	Lys	Ala	Asp	Arg	Lys 330	Phe	Leu	Asp	Lys	Ala 335	Leu	
	gaa Glu	gat Asp	tac Tyr	cgc Arg 340	gac Asp	gcc Ala	cgc Arg	aaa Lys	ggg Gly 345	ctg Leu	gac Asp	gat Asp	tta Leu	gct Ala 350	aaa Lys	ccg Pro	1056
	agc Ser	gag Glu	aaa Lys 355	gcc Ala	att Ile	cac His	ccg Pro	caa Gln 360	tat Tyr	ctg Leu	gcg Ala	cag Gln	caa Gln 365	att Ile	agt Ser	cat His	1104
	ttt Phe	gcc Ala 370	gcc Ala	gat Asp	gac Asp	gct Ala	att Ile 375	ttc Phe	acc Thr	tgt Cys	gac Asp	gtt Val 380	ggt Gly	acg Thr	cca Pro	acg Thr	1152
	gtg Val 385	tgg Trp	gcg Ala	gca Ala	cgt Arg	tat Tyr 390	cta Leu	aaa Lys	atg Met	aac Asn	ggc Gly 395	aag Lys	cgt Arg	cgc Arg	ctg Leu	tta Leu 400	1200
	ggt Gly	tcg Ser	ttt Phe	aac Asn	cac His 405	ggt Gly	tcg Ser	atg Met	gct Ala	aac Asn 410	gcc Ala	atg Met	ccg Pro	cag Gln	gcg Ala 415	ctg Leu	1248
	ggt Gly	gcg Ala	cag Gln	gcg Ala 420	aca Thr	gag Glu	cca Pro	gaa Glu	cgt Arg 425	cag Gln	gtg Val	gtc Val	gcc Ala	atg Met 430	tgc Cys	ggc Gly	1296
27									ggc Gly								1344
<i>5</i> 0									gtc Val								1392
									ggt Gly								1440
	gaa Glu	cta Leu	cac His	gac Asp	aca Thr 485	aac Asn	ttt Phe	gcc Ala	cgc Arg	att Ile 490	gcc Ala	gaa Glu	gcg Ala	tgc Cys	ggc Gly 495	att Ile	1488
									tct Ser 505								1536
		acc	ttc	t.cc	atc	gac	aat	ccg	gtg	ttg	gtg	gat	gtg	gtg	gtc	qcc	1584
	cgc Arg	Ala	Phe 515	Ser	Ile	Āsp	Ğİy	Pro 520	Val	Leu	Val	Asp	Val 525	Val	Val	Āla	

Lys Glu Glu Leu Ala Ile Pro Pro Gln Ile Lys Leu Glu Gln Ala Lys ggt ttc agc ctg tat atg ctg cgc gca atc atc agc gga cgc ggt gat 1680 Gly Phe Ser Leu Tyr Met Leu Arg Ala Ile Ile Ser Gly Arg Gly Asp 550 gaa gtg atc gaa ctg gcg aaa aca aac tgg cta agg taa 1719 Glu Val Ile Glu Leu Ala Lys Thr Asn Trp Leu Arg 570

<210> 2

<211> 572

<212> PRT

<213> Escherichia coli

<400> 2

Met Lys Gln Thr Val Ala Ala Tyr Ile Ala Lys Thr Leu Glu Ser Ala 1 5 10 15

Gly Val Lys Arg Ile Trp Gly Val Thr Gly Asp Ser Leu Asn Gly Leu 20 25 30

Ser Asp Ser Leu Asn Arg Met Gly Thr Ile Glu Trp Met Ser Thr Arg $\frac{35}{40}$ 45

His Glu Glu Val Ala Ala Phe Ala Ala Gly Ala Glu Ala Gln Leu Ser 50 55 60

Gly Glu Leu Ala Val Cys Ala Gly Ser Cys Gly Pro Gly Asn Leu His 70 75 80

Leu Ile Asn Gly Leu Phe Asp Cys His Arg Asn His Val Pro Val Leu 85 90 95

Ala Ile Ala Ala His Ile Pro Ser Ser Glu Ile Gly Ser Gly Tyr Phe 100 105 110

- Gln Glu Thr His Pro Gln Glu Leu Phe Arg Glu Cys Ser His Tyr Cys 115 120 125
- Glu Leu Val Ser Ser Pro Glu Gln Ile Pro Gln Val Leu Ala Ile Ala 130 135 140
- Met Arg Lys Ala Val Leu Asn Arg Gly Val Ser Val Val Val Leu Pro 145 150 155 160
- Gly Asp Val Ala Leu Lys Pro Ala Pro Glu Gly Ala Thr Met His Trp 165 170 175
- Tyr His Ala Pro Gln Pro Val Val Thr Pro Glu Glu Glu Glu Leu Arg 180 185 190
- Lys Leu Ala Gln Leu Leu Arg Tyr Ser Ser Asn Ile Ala Leu Met Cys 195 200 205
- Gly Ser Gly Cys Ala Gly Ala His Lys Glu Leu Val Glu Phe Ala Gly 210 215 220
- Lys Ile Lys Ala Pro Ile Val His Ala Leu Arg Gly Lys Glu His Val 225 230 235 240
- Glu Tyr Asp Asn Pro Tyr Asp Val Gly Met Thr Gly Leu Ile Gly Phe
 245 250 255
 - Ser Ser Gly Phe His Thr Met Met Asn Ala Asp Thr Leu Val Leu Leu 260 265 270
 - Gly Thr Gln Phe Pro Tyr Arg Ala Phe Tyr Pro Thr Asp Ala Lys Ile 275 280 285
 - Ile Gln Ile Asp Ile Asn Pro Ala Ser Ile Gly Ala His Ser Lys Val 290 295 300
 - Asp Met Ala Leu Val Gly Asp Ile Lys Ser Thr Leu Arg Ala Leu Leu 305 310 315 320

Pro Leu Val Glu Glu Lys Ala Asp Arg Lys Phe Leu Asp Lys Ala Leu 325 330 335

Glu Asp Tyr Arg Asp Ala Arg Lys Gly Leu Asp Asp Leu Ala Lys Pro 340 345 350

Ser Glu Lys Ala Ile His Pro Gln Tyr Leu Ala Gln Gln Ile Ser His 355 360 365

Phe Ala Ala Asp Asp Ala Ile Phe Thr Cys Asp Val Gly Thr Pro Thr 370 375 380

Val Trp Ala Ala Arg Tyr Leu Lys Met Asn Gly Lys Arg Arg Leu Leu 385 390 395 400

Gly Ser Phe Asn His Gly Ser Met Ala Asn Ala Met Pro Gln Ala Leu 405 410 415

Gly Ala Gln Ala Thr Glu Pro Glu Arg Gln Val Val Ala Met Cys Gly
420 425 430

Asp Gly Gly Phe Ser Met Leu Met Gly Asp Phe Leu Ser Val Val Gln 435 440 445

Met Lys Leu Pro Val Lys Ile Val Val Phe Asn Asn Ser Val Leu Gly
450
455
460

Phe Val Ala Met Glu Met Lys Ala Gly Gly Tyr Leu Thr Asp Gly Thr 465 470 475 480

Glu Leu His Asp Thr Asn Phe Ala Arg Ile Ala Glu Ala Cys Gly Ile 485 490 495

Thr Gly Ile Arg Val Glu Lys Ala Ser Glu Val Asp Glu Ala Leu Gln 500 505 510

Arg Ala Phe Ser Ile Asp Gly Pro Val Leu Val Asp Val Val Val Ala 515 520 525

Lys Glu Glu Leu Ala Ile Pro Pro Gln Ile Lys Leu Glu Gln Ala Lys 530 535 540

Gly Phe Ser Leu Tyr Met Leu Arg Ala Ile Ile Ser Gly Arg Gly Asp 545 550 550

Glu Val Ile Glu Leu Ala Lys Thr Asn Trp Leu Arg 565 570

<210> 3

<211> 1454

<212> DNA

<213> Escherichia coli

<220>

<221> misc feature

<222> (1)..(1454)

<223> mutagenic DNA

2 Int

<220>

<221> misc_feature

<222> (1)..(56)

<223> technical DNA/residue of the polylinker sequence

<220>

<221> misc feature

<222> (57)..(577)

<223> part of the 5' region (poxB1) of the poxB gene

```
<220>
<221>
      misc feature
<222>
      (578)..(646)
       technical DNA/residue of the polylinker sequence
<223>
<220>
<221>
       misc feature
<222>
       (647)..(1398)
<223>
      part of the 3' region (poxB2) of the poxB gene
<220>
<221> misc feature
      (1399)..(1454)
<222>
<223>
       technical DNA/residue of the polylinker sequence
```



<400> 60 ctagatgcat gctcgagcgg ccgccagtgt gatggatatc tgcagaattc gcccttctga acggtcttag tgacagtctt aatcgcatgg gcaccatcga gtggatgtcc acccgccacg 120 180 aagaagtggc ggcctttgcc gctggcgctg aagcacaact tagcggagaa ctggcggtct 240 gcgccggatc gtgcggcccc ggcaacctgc acttaatcaa cggcctgttc gattgccacc gcaatcacgt tccggtactg gcgattgccg ctcatattcc ctccagcgaa attggcagcg 300 360 gctatttcca ggaaacccac ccacaagagc tattccgcga atgtagtcac tattgcgagc 420 tggtttccag cccggagcag atcccacaag tactggcgat tgccatgcgc aaagcggtgc 480 ttaaccgtgg cgtttcggtt gtcgtgttac caggcgacgt ggcgttaaaa cctgcgccag 540 aaggggcaac catgcactgg tatcatgcgc cacaaccagt cgtgacgccg gaagaagaag 600 agttacgcaa actggcgcaa ctgctgcgtt attccaggcc taagggcgaa ttccagcaca 660 ctggcggccg ttactagtgg atccgagatc tgcagaattc gcccttctgc gtgcattgct

tccattggtg gaagaaaaag ccgatcgcaa gtttctggat aaagcgctgg aagattaccg 720 780 cgacgcccgc aaagggctgg acgatttagc taaaccgagc gagaaagcca ttcacccgca 840 atatctqqcq caqcaaatta gtcattttqc cgccgatgac gctattttca cctgtgacgt 900 tggtacgcca acggtgtggg cggcacgtta tctaaaaatg aacggcaagc gtcgcctgtt aggttcgttt aaccacggtt cgatggctaa cgccatgccg caggcgctgg gtgcgcaggc 960 1020 gacagagcca gaacgtcagg tggtcgccat gtgcggcgat ggcggtttta gcatgttgat gggcgatttc ctctcagtag tgcagatgaa actgccagtg aaaattgtcg tctttaacaa 1080 caqcqtqctq qqctttqtqq cqatqqaqat gaaagctggt ggctatttga ctgacggcac 1140 cgaactacac gacacaaact ttgcccgcat tgccgaagcg tgcggcatta cgggtatccg 1200 tgtagaaaaa gcgtctgaag ttgatgaagc cctgcaacgc gccttctcca tcgacggtcc 1260 ggtgttggtg gatgtggtgg tcgccaaaga agagttagcc attccaccgc agatcaaact 1320 cgaacaggcc aaaggtttca gcctgtatat gctgcgcgca atcatcagcg gacgcggtga 1380 1440 tqaaqtqatc qaactqqcaa qqqcqaattc caqcacactq gcggccgtta ctagtggatc 1454 cgagctcggt acca

<210> 4

<211> 720

<212> DNA

<213> Escherichia coli

als .

<220>

<221> misc feature

<222> (1)..(3)

<223> start codon of the delta poxB allele

<220>

<221> misc feature

```
<222> (1)..(605)
<223> 5' region of the delta poxB allele

<220>
<221> misc_feature
<222> (606)..(674)
<223> technical DNA/residues of the polylinker sequence
<220>
```

<221> misc feature

<222> (675)..(1445)

<223> 3' region of the delta poxB allele

<220>

<221> misc feature

<222> (1446)..(1448)

<223> stop codon of the delta poxB allele

CM

<400> atgaaacaaa cggttgcagc ttatatcgcc aaaacactcg aatcggcagg ggtgaaacgc 60 atctggggag tcacaggcga ctctctgaac ggtcttagtg acagtcttaa tcgcatgggc 120 accategagt ggatgtecae eegecaegaa gaagtggegg cetttgeege tggegetgaa 180 gcacaactta gcggagaact ggcggtctgc gccggatcgt gcggccccgg caacctgcac 240 ttaatcaacg gcctgttcga ttgccaccgc aatcacgttc cggtactggc gattgccgct 300 catattccct ccagcgaaat tggcagcggc tatttccagg aaacccaccc acaagagcta 360 ttccgcgaat gtagtcacta ttgcgagctg gtttccagcc cggagcagat cccacaagta 420 ctggcgattg ccatgcgcaa agcggtgctt aaccgtggcg tttcggttgt cgtgttacca 480

ggcgac	gtgg	cgttaaaacc	tgcgccagaa	ggggcaacca	tgcactggta	tcatgcgcca	540
caacca	gtcg	tgacgccgga	agaagaagag	ttacgcaaac	tggcgcaact	gctgcgttat	600
tccagg	ccta	agggcgaatt	ccagcacact	ggcggccgtt	actagtggat	ccgagatctg	660
cagaat	tcgc	ccttctgcgt	gcattgcttc	cattggtgga	agaaaaagcc	gatcgcaagt	720
<210>	5						
<211>	20						
<212>	DNA						
<213>	Arti	ficial Sequ	ience				
<220>							
<223>	Synt	hetic DNA					
<400> ctgaac		ttagtgacag					20
<210>	6						
<211>	24						
<212>	DNA						
<213> 1 yw/	Arti	ficial Sequ	ence				
<220>							
<223>	Synt	hetic DNA					
<400> aggccto	6 ggaa	taacgcagca	gttg				24
<210>	7						
<211>	21						
<212>	DNA						
<213×	7 r+ i ·	ficial Some	ongo				

<220>		
<223>	Synthetic DNA	
<400> ctgcgt	7 Egcat tgcttccatt g	21
<210>	8	
<211>	22	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Synthetic DNA	
<400> gccagt	8 toga toacttoato ac	22
<210>	9	
<211>	20	
<212>	DNA	
<213>	Artificial Sequence	
y w		
<220>		
<223>	Synthetic DNA	
<400> tgaaca	9 cttc tggcggtacg	20
<210>	10	
<211>	20	
<212>	DNA	

(213)	Artificial Sequence	
<220>		
<223>	Synthetic DNA	
	10 cgaa gctaatatgg	20
<210>	11	
<211>	20	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Synthetic DNA	
	11 gcat cggcgaggca	20
		20
<210>	12	
<211>	20	
<212>	DNA	
<213>	Artificial Sequence	
M		
<220>		
<223>	Synthetic DNA	
	12 gat ggcgatgacg	20
		_ 0